

## Biochemical content, minerals, and antioxidant activity of fruit jiaosu obtained by natural fermentation

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### Abstract

This study was conducted to determine the pH, protein concentration, mineral, phenolic, flavonoid, and organic acid contents, as well as the antioxidant activity of three batches of fruit jiaosu (F1B3, F1B4, and F1B5). The jiaosu was prepared by mixing ten types of fresh fruits, raw honey, and water (3:1:10, w/w/w), and fermented at room temperature for six months. All fruit jiaosu had low pH (2.96–3.69), and the protein concentration was ranged from 8.45 to 16.2 µg/mL. Potassium (605.0–657.2 mg/L) was found to be the most abundant mineral in all the samples, followed by magnesium, calcium, and sodium. The total phenolic and flavonoid contents were 183.1–210.5 µg gallic acid equivalent/mL and 45.2–53.8 µg quercetin equivalent/mL, respectively. Acetic acid was the major organic acid in the F1B4 and F1B5 samples (6.58 g/L and 20.7 g/L, respectively) while the F1B3 sample contained the highest amount of lactic acid (12.1 g/L). Ascorbic and citric acids were present in small amounts in all samples. All the samples showed stronger scavenging activity against 2,2-diphenyl-1-picrylhydrazyl radicals (half-maximum inhibitory concentration, IC<sub>50</sub>: 13.6–16.8% v/v) than against hydrogen peroxide (IC<sub>50</sub>: 47.2–50.6% v/v). The ferric reducing antioxidant power and oxygen radical absorbance capacity values for the samples were 14.9–20.9 µg ascorbic acid equivalent/mL and 23.9–25.4 mg Trolox equivalent/mL, respectively. The results indicated that the fruit jiaosu is a complex acidic solution containing proteins, minerals, phenolic compounds, and organic acids with antioxidant activities. Natural fermentation may cause variations in the organic acid content of the fruit jiaosu.

## 1. Introduction

Jiaosu is the bioactive liquid product obtained after fermentation of materials from animals, plants, or mushrooms by various microorganisms. According to the China Biotech Fermentation Industry Association (2020), jiaosu can be classified based on the product applications into five types, which are edible jiaosu, environmental jiaosu, daily chemical products jiaosu, fodder jiaosu, and agricultural jiaosu. Edible jiaosu, also known as fermented juice or enzyme drink conventionally, is usually produced by fermenting single or few fruits and vegetables at room temperature for at least three months before it is ready for consumption. A starter culture such as lactic acid bacteria or an additional carbon source such as honey or brown sugars can be

added into the fermentation process. This beverage has gained popularity in Asia, especially in East Asian countries.

Fermented juices have been reported to possess many health-promoting activities. *Lactobacillus plantarum*-containing fermented cabbage-apple juice is able to reduce cholesterol levels in the rats fed with a high-fat and high-cholesterol diet (Jeon *et al.*, 2019). Some fermented fruit or vegetable juices, such as madan fruit (*Garcinia schomburgkiana*), bitter melon (*Momordica charantia*), and noni (*Morinda citrifolia*) have been shown to have antioxidant and antidiabetic effects (Park *et al.*, 2017; Simamora *et al.*, 2019; Thummajitsakul *et al.*, 2019). Fermented beetroot juice has been reported to possess a cytotoxic effect against

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human liver cancer cells (Vaithilingam *et al.*, 2016). Moreover, the daily intake of fermented citrus juice could alleviate perennial allergic rhinitis (Harima-Mizusawa *et al.*, 2016).

As most of the studies have focused on a single fruit or vegetable for fermentation, little is known about the biochemical content and antioxidant activity of jiaosu when a mixture of fruits is used. This study was thus conducted to evaluate the pH, protein, mineral, phenolic, flavonoid, and organic acid contents, as well as the antioxidant activity of three different batches of fruit jiaosu. In this study, a mixture of 10 types of fresh fruits (apple, banana, guava, honeydew, lemon, orange, papaya, pineapple, starfruit, and watermelon) was used during the fermentation process.

## 2. Materials and methods

### 2.1 Preparation of fruit jiaosu

Three batches of fruit jiaosu (F1B3, F1B4, and F1B5) were provided by EN-Nature Sdn. Bhd. (Penang, Malaysia) for the study. The suspended solids in the fruit jiaosu samples (Figure 1a) were removed by centrifugation at  $3850\times g$  for 10 mins at room temperature. The supernatant was used for analysis. Each batch of samples was analysed in triplicate. According to the manufacturer, the fruit jiaosu was prepared by mixing 10 types of fresh fruits (apple, banana, guava, honeydew, lemon, orange, papaya, pineapple, starfruit, and watermelon), raw honey from honey bees, and filtered drinking water at a ratio of 3:1:10 (w/w/w) and left fermented naturally in a 160 L food-grade high-density polyethylene drum (Figure 1b) at room temperature for six months. The composition of the fresh fruits followed the formulation of the manufacturer and was the same for the three batches.

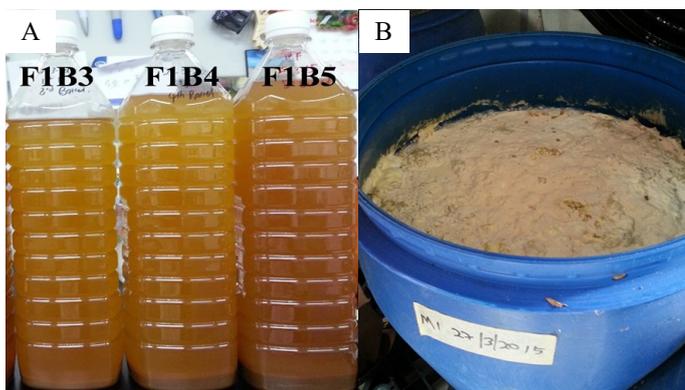


Figure 1. Fruit jiaosu. (a) Three batches, F1B3, F1B4, and F1B5 were used in the study. (b) The fermentation is carried out in a 160-L food-grade high-density polyethylene drum at room temperature for six months.

### 2.2 pH and protein concentration

The pH of each sample was measured using a

calibrated pH meter (Model PB-10, Sartorius Lab Instruments GmbH and Co. KG, Goettingen, Germany). The protein concentration was determined using the Bradford protein assay (Bradford, 1976). Different concentrations of bovine serum albumin (2.5, 5, 10, 15, 20, and 25  $\mu\text{g}/\text{mL}$ ) were used to generate a protein standard curve. One mL of protein standard or sample was mixed with 1 mL of Bradford reagent and incubated at room temperature for 10 mins. The absorbance value was then read at 595 nm using an ultraviolet-visible (UV-Vis) spectrophotometer (Genesys™ 10S, Thermo Fisher Scientific Inc., Waltham, MA, USA). The linear regression equation for the protein standard curve was  $y = 0.0206x + 0.1349$  with a regression coefficient ( $R^2$ ) of 0.9898, whereby y is absorbance value and x is the concentration of bovine serum albumin.

### 2.3 Total phenolic content (TPC) and total flavonoid content (TFC)

The TPC and TFC were determined based on the Folin-Ciocalteu method and aluminium chloride method, respectively (Herald *et al.*, 2012). Gallic acid (5–320  $\mu\text{g}/\text{mL}$ ) and quercetin (25–500  $\mu\text{g}/\text{mL}$ ) were used to construct standard curves for TPC and TFC, respectively, while deionised water was used as a negative control for both assays. For the TPC assay, 25  $\mu\text{L}$  of sample/standard, 75  $\mu\text{L}$  of deionised water, and 25  $\mu\text{L}$  of 50% Folin-Ciocalteu's phenol reagent was mixed and shaken for 6 mins. The mixture was then added with 100  $\mu\text{L}$  of 700 mmol/L sodium carbonate and incubated in the dark at room temperature for 90 mins. For the TFC assay, a mixture containing 25  $\mu\text{L}$  of sample/standard, 10  $\mu\text{L}$  of 725 mmol/L sodium nitrite, 100  $\mu\text{L}$  of deionised water, and 15  $\mu\text{L}$  of 750 mM aluminium chloride were shaken for 6 mins. After that, 50  $\mu\text{L}$  of 1 mol/L sodium hydroxide was added and the mixture was made up to 250  $\mu\text{L}$  with deionised water. The microplate was incubated for 60 mins in the dark at room temperature. The absorbance values for TPC and TFC were measured at 765 and 420 nm, respectively, using a microplate reader (FLUOstar® Omega, BMG Labtech, Mornington, VIC, Australia). The TPC and TFC values were expressed as  $\mu\text{g}$  gallic acid equivalent/mL sample and  $\mu\text{g}$  quercetin equivalent/mL sample, respectively. The linear regression equation for the gallic acid standard curve was  $y = 0.0067x + 0.0164$  ( $r^2 = 0.9996$ ), and for the quercetin standard curve was  $y = 0.0007x - 0.0004$  ( $r^2 = 0.9972$ ). For each equation, y represents the absorbance value and x represent the concentration of the standard (gallic acid/quercetin).

### 2.4 Mineral content

The mineral content (Ca, Mg, K, Na, Al, Co, Cu, Cr,

Fe, Mn, Ni, Se, Zn, Rb, Sr, Ag, Ba, Pb, Bi, As, and Cd) was quantified using an inductively coupled plasma-mass spectrometric (ICP-MS) method (Dehelean and Magdas, 2013). The plasma (argon), auxiliary, and nebuliser gases of the ICP-MS (NexION<sup>®</sup> 300, PerkinElmer Inc., Waltham, MA, USA) was set at 16.0, 1.20, and 0.98 L/min, respectively. Each sample was filtered using a 0.45 µm nylon membrane syringe filter and diluted 1000 folds with ultrapure water prior to analysis. Standard curves were generated using a multi-elements calibration standard solution (100–500 µg/L) for quantification of the minerals in the samples.

## 2.5 Organic acid content

The organic acid content (malic, ascorbic, lactic, acetic, citric, and fumaric acids) was assayed using a modified reversed phase-high performance liquid chromatographic (RP-HPLC) method (Kordis-Krapez *et al.*, 2001). The RP-HPLC (Model 1100, Agilent Technologies Inc., Santa Clara, CA, USA) was equipped with a column oven (30°C) and a UV-Vis variable wavelength detector set at 210 nm. The separation of organic acids was achieved using a C18 column (10 µm, 250 × 4 mm i.d.; Luna, Phenomenex Inc., Torrance, CA, USA). The mobile phase was 100% of 20 mmol/L sodium phosphate buffer (pH 2.5) running at isocratic mode, and the flow rate was 0.5 mL/min. Each sample was diluted 10 folds with 20 mmol/L sodium phosphate buffer (pH 2.5) prior to injection. The injection volume was 20 µL. Five concentrations of standard mixtures of malic (200–800 mg/L), ascorbic (20–80 mg/L), lactic (200–800 mg/L), acetic (200–800 mg/L), citric (200–800 mg/L), and fumaric (2–8 mg/L) acids were prepared for the standard curve of individual organic acid. The linear regression equations for the standard curves were  $y = 1.8116x - 8.7188$  ( $R^2 = 0.9998$ ) for malic acid,  $y = 22.823x - 20.242$  ( $R^2 = 0.9994$ ) for ascorbic acid,  $y = 1.0716x - 4.8765$  ( $R^2 = 0.9997$ ) for lactic acid,  $y = 1.0994x - 7.7218$  ( $R^2 = 0.9996$ ) for acetic acid,  $y = 2.1446x - 7.9540$  ( $R^2 = 0.9999$ ) for citric acid, and  $y = 335.56x - 24.181$  ( $R^2 = 0.9996$ ) for fumaric acid, whereby  $y$  = peak area (mAU·min) of respective organic acids and  $x$  = concentration of respective organic acids.

## 2.6 Antioxidant activity

### 2.6.1 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH radical scavenging activity was measured using the method of Brand-Williams *et al.* (1995) with slight modifications. Each sample was evaluated at seven concentrations, 0.1, 1, 5, 10, 20, 30, and 40% v/v. One mL of sample was added into 3 mL of 0.1 mmol/L DPPH solution, mixed well, and allowed to stand in the dark at room temperature for 30 mins. The absorbance

was measured at 517 nm using the UV-Vis spectrophotometer. Distilled water was used as a blank while ascorbic acid (1–100 µg/mL) was used as a standard. The percentage of DPPH radical scavenging activity was calculated using the formula  $[(A_0 - A_1)/A_0] \times 100$ , whereby  $A_0$  is the absorbance of blank, and  $A_1$  is the absorbance in the presence of the sample or ascorbic acid. A plot of the percentage of DPPH radical scavenging activity against the concentration of sample or ascorbic acid was prepared to determine the half-maximum inhibitory concentration ( $IC_{50}$ ).

### 2.6.2 Hydrogen peroxide ( $H_2O_2$ ) scavenging assay

The  $H_2O_2$  scavenging assay was performed using the method of Ruch *et al.* (1989) with slight modifications. Approximately 500 µL of sample (10, 20, 30, 40, 50, 60, and 70% v/v) was added into 1 mL of 4 mmol/L  $H_2O_2$  prepared in 0.1 mol/L sodium phosphate buffer (pH 7.4), mixed well, and allowed to stand in the dark at room temperature for 10 mins. The absorbance of  $H_2O_2$  at 230 nm was measured using the UV-Vis spectrophotometer. Seven concentrations of ascorbic acid (0.4, 0.6, 0.8, 1.0, 1.2, 1.4, and 1.6 mg/mL) were prepared for the standard curve. For the blank solutions, the  $H_2O_2$  was replaced with 1 mL of 0.1 mol/L sodium phosphate buffer (pH 7.4). The percentages of  $H_2O_2$  scavenged by the samples and ascorbic acid were calculated from the formula  $[(A_0 - A_1)/A_0] \times 100$ , whereby  $A_0$  is the absorbance of the blank solution, and  $A_1$  is the absorbance of the sample or ascorbic acid. The inhibition curves were constructed and  $IC_{50}$  values were obtained.

### 2.6.3 Ferric-reducing antioxidant power (FRAP) assay

The FRAP assay was performed according to Benzie and Strain (1996) with slight modifications. A 0.1 mL of ferrous sulphate standard solution (0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mmol/L) or 1 mL of sample (1, 5, 10, 20, 30, 40, and 50% v/v) was added into 3 mL of FRAP reagent and incubated at 37°C for 10 mins. The freshly prepared FRAP reagent contained 10 mmol/L 2,4,6-tripyridyl-*s*-triazine solution, 20 mmol/L ferric chloride solution, and 300 mmol/L acetate buffer (pH 3.6) at a ratio of 1:1:10 (v/v/v). Ascorbic acid (1, 2, 4, 6, 8, 10, and 12 µg/mL) was used as a positive control. The absorbance of the samples or ascorbic acid was measured at 593 nm using the UV/Vis spectrophotometer with distilled water as a blank. Absorbances obtained for the samples were interpolated from the ferrous sulphate standard curve as FRAP values (mmol  $Fe^{2+}$ /mL), and further expressed as µg ascorbic acid equivalent/mL sample based on the ascorbic acid standard curve. The linear regression equation for the ferrous sulphate standard curve was  $y = 0.6912x - 0.0164$  ( $R^2 = 0.9998$ ) with  $y$  = absorbance

value at 593 nm and  $x$  = concentration of ferrous sulphate. While for the ascorbic acid standard curve, the linear regression equation of the curve was  $y = 0.1151x + 0.0762$  with an  $R^2$  value of 0.9952, whereby  $y$  is FRAP value and  $x$  is the concentration of ascorbic acid.

#### 2.6.4 Oxygen radical absorbance capacity (ORAC) assay

The ORAC of each sample was assayed using a fluorometric method, as described previously (Heng et al., 2020). The six concentrations (0.03, 0.06, 0.13, 0.25, 0.50, and 1.00% v/v) were prepared for each sample in 75 mmol/L sodium phosphate buffer (pH 7.0). Trolox with a concentration range of 39–313  $\mu\text{g/mL}$  was used for constructing a standard curve. The sodium phosphate buffer was used as a blank. After the addition of 2,2-azobis(2-methylpropionamide) dihydrochloride (peroxyl radical inducer), the fluorescence intensity of the 96-well black microplate was monitored at 37°C every 90 s for 40 cycles. The area under the fluorescence decay curve (AUC) for each concentration of the sample or Trolox was subtracted with the AUC of blank to obtain the net AUC value. The standard curve of Trolox was plotted using the net AUC value ( $y$ ) against concentration ( $x$ ). The linear regression equation for the curve was  $y = 295.96x + 3.7062$  with an  $R^2$  value of 0.9920. The ORAC value of each sample was then determined from the plot using the sample's net AUC value and expressed as mg Trolox equivalent/mL sample after normalisation of the sample volume used.

#### 2.7 Data analysis

All the data were presented in mean  $\pm$  standard deviation of three replicates. The data were examined for statistical significance ( $p < 0.05$ ) using the IBM SPSS Statistics for Windows Version 22.0 software (IBM Corp., Armonk, USA). Student's  $t$ -test and one-way analysis of variance were used for the analysis while Tukey's HSD test or Dunnett's T3 test was used for post hoc multiple comparisons.

### 3. Results

#### 3.1 pH, protein concentration, total phenolic and flavonoid contents

All the fruit jiaosu samples were acidic with pH

values ranging from 2.96 to 3.69. The F1B5 batch had a lower pH value ( $p < 0.05$ ) compared with the other two batches. The protein concentrations of the samples were 8.45–16.2  $\mu\text{g/mL}$  (Table 1). The F1B5 batch showed the lowest protein concentration ( $p < 0.05$ ), approximately half that of the F1B3 and F1B4 batches. The fruit jiaosu samples were high in phenolic compounds. The highest TPC was shown by the F1B5 batch, followed by the F1B3 and F1B4 batches (Table 1). On the other hand, the F1B4 and F1B5 batches had similar TFC but higher than that of the F1B3 batch. The results indicated that non-flavonoid phenolic compounds may present in these samples.

#### 3.2 Mineral content

Potassium (605.0–657.2 mg/L) was found to be the major mineral present in the fruit jiaosu samples, followed by Mg (41.2–47.0 mg/L), Ca (17.5–27.7 mg/L), Na (2.3–5.3 mg/L), and Mn (0.2–2.5 mg/L) (Figure 2). The concentrations of these minerals were not significantly different among the batches, except for the F1B5 batch which contained significantly higher ( $p < 0.05$ ) Mg concentration than the other two batches. Other minerals detected were Ni, Cr, Co, Cu, Zn, Se, Rb, and Sr, and their concentrations were all  $< 1$  mg/L. Al, Fe, Ag, Ba, Pb, Bi, As, and Cd were not detected in any of the samples.

#### 3.3 Organic acid content

Acetic acid was the major organic acid in the F1B4 and F1B5 batches while lactic acid was the major organic acid in the F1B3 batch (Table 2). The F1B5 batch had the highest acetic acid content, approximately 2.3- and 3.2-fold higher than that in the F1B3 and F1B4 batches, respectively. A similar trend was also observed for the citric acid concentration in the F1B5 batch, approximately 1.8- and 2.0-fold higher than that in the F1B3 and F1B4 batches, respectively. In contrast, the F1B4 sample had significantly lower ( $p < 0.05$ ) lactic acid content (0.27 g/L) compared to the other two batches ( $> 12$  g/L). All the batches had similar ascorbic acid concentration. Malic acid was not detected in any of the samples while fumaric acid was only present in a minute amount in the F1B3 batch. Some unknown compounds, probably organic acids too were detected in the F1B4 and F1B5 samples (Figure 3).

Table 1. pH, protein concentration, total phenolic content, and total flavonoid content of different batches of fruit jiaosu

Sample	pH	Protein concentration ( $\mu\text{g/mL}$ )	Total phenolic content ( $\mu\text{g}$ gallic acid equivalent/mL)	Total flavonoid content ( $\mu\text{g}$ quercetin equivalent/mL)
F1B3	3.35 $\pm$ 0.01 <sup>a</sup>	16.2 $\pm$ 0.3 <sup>a</sup>	202.4 $\pm$ 0.4 <sup>a</sup>	45.2 $\pm$ 3.3 <sup>a</sup>
F1B4	3.69 $\pm$ 0.02 <sup>b</sup>	15.2 $\pm$ 0.1 <sup>b</sup>	183.1 $\pm$ 2.6 <sup>b</sup>	53.8 $\pm$ 1.7 <sup>b</sup>
F1B5	2.96 $\pm$ 0.01 <sup>c</sup>	8.45 $\pm$ 0.05 <sup>c</sup>	210.5 $\pm$ 1.2 <sup>c</sup>	53.2 $\pm$ 3.6 <sup>b</sup>

Values are expressed as mean  $\pm$  SD,  $n = 3$ . Values with different superscript within a column are significantly different ( $p < 0.05$ ) by one-way analysis of variance.

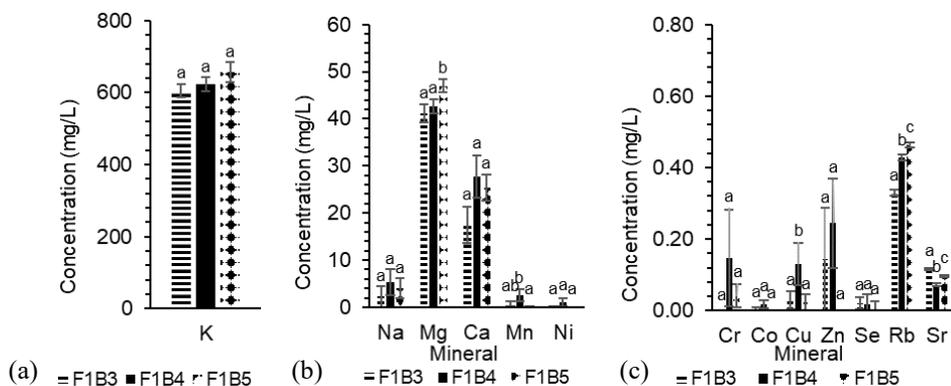


Figure 2. The mineral content of different batches of fruit jiaosu samples as quantified using inductively coupled plasma-mass spectrometry. (a) Potassium; (b) Sodium, magnesium, calcium, manganese, and nickel; (c) Other minor minerals. Bars are expressed as mean±standard deviation of three replicates. Bars notated with different alphabetical letters are significantly difference ( $p<0.05$ ) by one-way analysis of variance.

Table 2. Organic acid content of different batches of fruit jiaosu

Sample	Concentration (g/L)					
	Malic acid	Ascorbic acid	Lactic acid	Acetic acid	Citric acid	Fumaric acid
F1B3	ND	0.017±0.003 <sup>a</sup>	12.1±0.6 <sup>b</sup>	8.97±0.77 <sup>b</sup>	0.53±0.06 <sup>a</sup>	0.004±0.0003
F1B4	ND	0.020±0.002 <sup>a</sup>	0.27±0.08 <sup>a</sup>	6.58±0.27 <sup>a</sup>	0.48±0.04 <sup>a</sup>	ND
F1B5	ND	0.018±0.003 <sup>a</sup>	14.1±0.2 <sup>c</sup>	20.7±0.01 <sup>c</sup>	0.95±0.01 <sup>b</sup>	ND

ND: not detected. Values are expressed as mean±SD,  $n = 3$ . Values with different superscript within a column are significantly different ( $p<0.05$ ) by one-way analysis of variance.

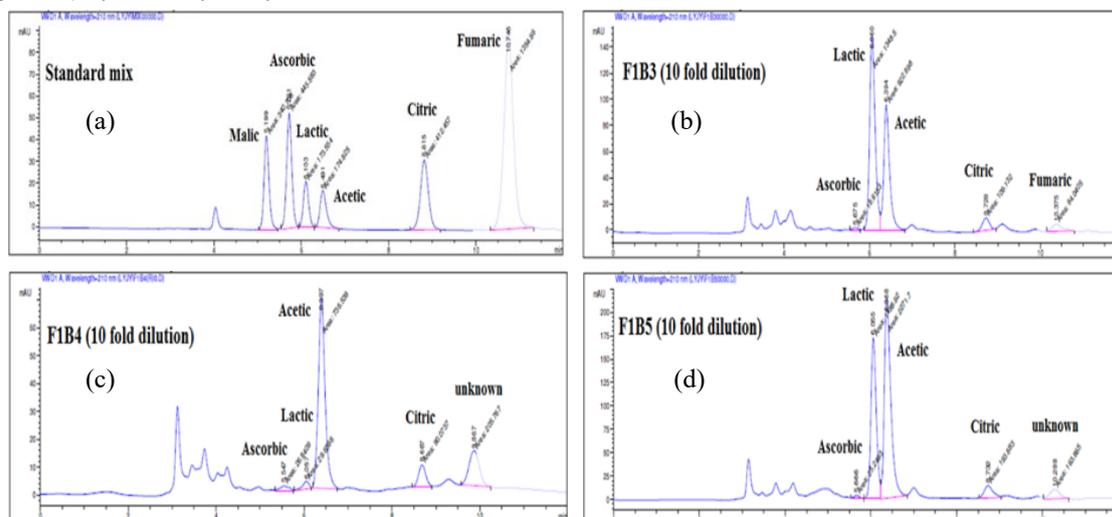


Figure 3. Chromatograms of standard organic acids (a) and organic acids in different batches of fruit jiaosu samples (b-d) obtained using a reversed phase-high performance liquid chromatography. Column: Luna C18, 250 x 4 mm, 10  $\mu$ m; Isocratic mobile phase: 20 mmol/L sodium phosphate buffer (100%), pH 2.5; Flow rate: 0.5 mL/min; Injection volume: 20  $\mu$ L; Column temperature: 30°C; Detection wavelength: 210 nm.

### 3.4 Antioxidant activity

Four assays were used to assess the antioxidant activity of fruit jiaosu samples. All the samples showed DPPH radical scavenging activity and  $H_2O_2$  scavenging activity in a concentration-dependent manner (Figure 4). Higher concentrations of jiaosu samples were needed to scavenge  $H_2O_2$  compared with DPPH radicals, which was also reflected by their significant ( $p<0.001$ ) differences of  $IC_{50}$  values (Table 3). The FRAP for the three batches of samples were also significantly different ( $p<0.05$ ), with the values ranging from 14.9 to 20.9  $\mu$ g ascorbic acid equivalent/mL. The F1B3 batch showed the lowest  $IC_{50}$  value for DPPH radical scavenging

activity and the highest FRAP value while the F1B5 batch had the lowest  $IC_{50}$  for  $H_2O_2$  scavenging activity (Table 3). However, all the samples showed similar antioxidant activity when they were assessed using the ORAC assay.

## 4. Discussion

The pH, protein, phenolic, and organic acid contents were significantly varied among the three batches, which is likely to have resulted from the natural fermentation process that occurred in the samples. The presence of organic acids, especially lactic and acetic acids, signifies the low pH values of the fruit jiaosu samples. The results

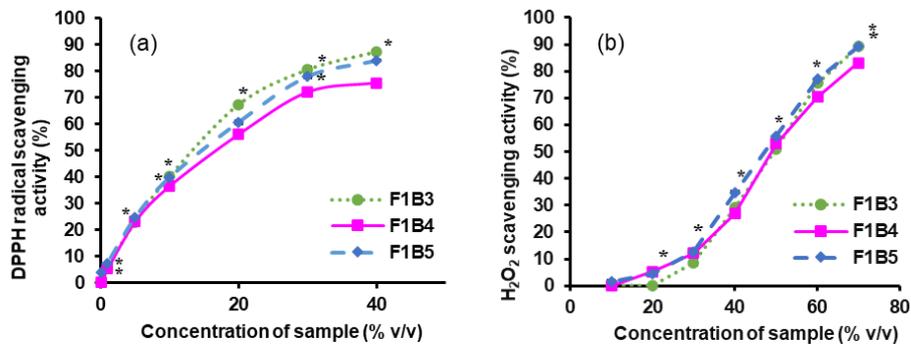


Figure 4. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (a) and hydrogen peroxide ( $H_2O_2$ ) (b) scavenging activity of different batches of fruit jiaosu. Values are expressed as mean $\pm$ standard deviation of three replicates. Values notated with asterisk marks indicate significant differences ( $p < 0.05$ ) by one-way analysis of variance.

Table 3. Antioxidant activity of different batches of fruit jiaosu

Sample	IC <sub>50</sub> for DPPH radical scavenging activity (% v/v)	IC <sub>50</sub> for $H_2O_2$ scavenging activity (% v/v)	FRAP value ( $\mu$ g ascorbic acid equivalent/mL)	ORAC value (mg Trolox equivalent/mL)
F1B3	13.6 $\pm$ 0.3 <sup>a,*</sup>	48.9 $\pm$ 0.1 <sup>b</sup>	20.9 $\pm$ 0.7 <sup>c</sup>	23.9 $\pm$ 3.9 <sup>a</sup>
F1B4	16.8 $\pm$ 0.5 <sup>c,*</sup>	50.6 $\pm$ 0.1 <sup>c</sup>	16.4 $\pm$ 1.2 <sup>b</sup>	25.2 $\pm$ 2.5 <sup>a</sup>
F1B5	14.8 $\pm$ 0.2 <sup>b,*</sup>	47.2 $\pm$ 0.2 <sup>a</sup>	14.9 $\pm$ 0.7 <sup>a</sup>	25.4 $\pm$ 0.4 <sup>a</sup>
Ascorbic acid ( $\mu$ g/mL)	11	939	NA	NA

IC<sub>50</sub>: half-maximum inhibitory concentration; DPPH: 2,2-diphenyl-1-picrylhydrazyl;  $H_2O_2$ : hydrogen peroxide; FRAP: ferric-reducing antioxidant power; ORAC: oxygen radical absorbance capacity. NA: not applicable. Values are expressed as mean $\pm$ SD, n = 3. Values with different superscript within a column are significantly different ( $p < 0.05$ ) by one-way analysis of variance. Values with asterisk marks indicate significant differences ( $p < 0.001$ ) from  $H_2O_2$  scavenging activity using Student's *t*-test.

may also imply the involvement of lactic acid bacteria and/or acetic acid bacteria in the fermentation process. Lactic acid bacteria can ferment simple carbohydrates in the fruits and produce copious amounts of lactic acid either homofermentative (>95% lactic acid from glucose) or heterofermentative (producing acetic acid, ethanol, and carbon dioxide in addition to lactic acid) (Narvhus and Axelsson, 2003). While acetic acid bacteria are able to oxidise ethanol, which is usually produced by yeasts during the early stage of fermentation, into acetic acid, and they have high resistance to acetic acid released into the fermentative medium (Nakano and Fukaya, 2008; Gomes *et al.*, 2018). Ma *et al.* (2018) studied the bacterial microbiota of three commercial fermented fruit and vegetable juices and identified six species of bacteria, including *Lactobacillus* spp. (one of the members of lactic acid bacteria) as the core microbes in the juices.

The low pH of jiaosu tends to reduce the stability of proteins, and a high amount of acetic acid can denature proteins (Perlmann and Kaufman, 1949). This may explain why the F1B5 batch had the lowest pH value and the lowest protein concentration among the samples. The significant differences in lactic acid and acetic acid concentrations among the three batches suggest that the microorganism (bacteria and fungi) population involved in the fermentation process might be different in these samples (Ho *et al.*, 2018; Anal, 2019). Besides, the unknown peaks recorded in the chromatograms for the F1B4 and F1B5 batches imply the possibility of other

organic acids present in the fruit jiaosu samples.

The antioxidant activities of the fruit jiaosu are likely to be contributed by phenolic compounds due to the high TPC in the samples. Polyphenols and phenolic acids are the two major groups of plant phenolic compounds. Polyphenols can be divided into flavonoids and non-flavonoids, such as lignans, stilbenoids, tannins, quinones, and coumarins. Phenolic acids are carboxylic acids derived from either benzoic or cinnamic acid skeletons. The most abundant benzoic acid derivatives are *p*-hydroxybenzoic, vanillic, syringic, and gallic acids, while common cinnamic acid derivatives include *p*-coumaric, caffeic, ferulic, and sinapic acids (Neilson *et al.*, 2017). They are commonly found in vegetables and fruits (Ongphimai *et al.*, 2013; Lima *et al.*, 2014; Stafussa *et al.*, 2018). The presence of hydroxyl groups in the molecular structure of phenolic compounds enables them to function as radical scavengers, hydrogen donors, metal chelators, and reducing agents (Nimse and Pal, 2015). Besides phenolic compounds, organic acids such as ascorbic, lactic, and citric acids play an important role in the antioxidant activity of the fruit jiaosu. Ascorbate and lactate can scavenge free radicals such as hydroxyl radicals and superoxide anion (Groussard *et al.*, 1985; Beyer, 1994). Citric acid can chelate ferric and cupric ions, and such chelation helps to prevent lipid peroxidation in the human body (Mahoney and Graf, 1986; Frankel, 2005). Therefore, the antioxidant property of the fruit jiaosu is attributed to the phenolic compounds and organic acids present in the

solution.

## 5. Conclusion

Fruit jiaosu is a complex acidic solution containing proteins, minerals, phenolic compounds, and organic acids with antioxidant activity. Further work on the microbial population as well as other biological activities, such as antimicrobial and antidiabetic potential is necessary to gain a more comprehensive understanding of the content and health-promoting effects of fruit jiaosu.

## Conflict of interests

The authors declare that there is no conflict of interest associated with the content of this manuscript.

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